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SIMULTANEOUS DETERMINATION OF PROFENOFOS, DIAZINON, AND CHLORPYRIFOS IN TERNARY PESTICIDE MIXTURES USING MEAN CENTERING OF RATIO SPECTROPHOTOMETRY

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ABSTRACT
A separation-free UV–Vis spectrophotometric method via a mean-centered ratio spectra methodology (MCR) approach was developed and validated for the simultaneous determination of profenofos, diazinon, and chlorpyrifos in laboratory-prepared ternary pesticide mixtures. The method showed high analytical efficiency, featuring high linearity (r = 0.9973–0.9999), high recovery values (103.56–107.92%), and low sensitivity limits (LOD: 0.695–19.42 µg/mL; LOQ: 2.31–64.75 µg/mL). Sequential ratio transformations followed by mean-centering effectively resolved the substantial spectral overlap among the three organophosphates, enabling selective quantification without chromatographic separation. The validated MCR–UV–Vis procedure thus provides a rapid, solvent-efficient, and cost-effective alternative to traditional chromatographic techniques and is suitable for the routine determination of multicomponent pesticide mixtures.
Keywords: Mean Centering of Ratio (MCR), UV–Vis Spectrophotometry, Organophosphate Pesticides, Profenofos, Diazinon, Chlorpyrifos, Multiresidue Analysis

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INTRODUCTION

Excessive pesticide application can lead to significant residue accumulation[1], especially when applications are made shortly before harvest.[2,3] Organophosphate pesticides such as diazinon, chlorpyrifos, and profenofos are among the most frequently detected compounds in agricultural commodities, with reports of diazinon occasionally exceeding MRLs[4], chlorpyrifos being restricted to 0.01 mg/kg under EU regulations[5,6], and profenofos remaining detectable at 1.3–1.6 mg/kg even one day after application[7,8], although most samples still meet EFSA standards.[9] Residue monitoring typically relies on GC–MS/MS and LC–MS/MS due to their excellent sensitivity and selectivity.[10–13] The MCR-based ratio spectrophotometric technique presents a simple and effective analytical solution, a spectrophotometric approach capable of resolving overlapping UV–Vis spectra without chromatographic separation. Previous studies have demonstrated its accuracy and applicability in pesticide and environmental analyses.[14,15] However, no previous study has described the application of the MCR approach for the concurrent quantification of profenofos, diazinon, and chlorpyrifos in ternary mixtures, despite the considerable spectral overlap among these organophosphates and the analytical challenges this overlap creates. Their closely aligned UV–vis absorption bands make conventional spectrophotometric techniques insufficient without advanced mathematical treatment, underscoring the need for a selective and efficient alternative method. Accordingly, this study develops and validates an MCR–UV–Vis spectrophotometric procedure specifically tailored for accurate quantification of these three pesticides in laboratory-prepared multicomponent mixtures. By integrating sequential ratio transformations with mean-centering, the method enhances spectral resolution and enables reliable extraction of analyte-specific amplitudes, while

comprehensive validation covering linearity, precision, accuracy, sensitivity, and selectivity demonstrates its robustness and suitability as a practical, cost-effective alternative to chromatographic techniques for multiresidue pesticide analysis.

EXPERIMENTAL

Chemicals and Instruments

Standard profenofos, diazinon, and chlorpyrifos (Sigma Aldrich, USA) were used. Methanol (SmartLab) served as the solvent. UV spectra were recorded using Shimadzu UV-1800 (200–400 nm; slit width 1 nm; medium scan speed). MCR processing utilised MATLAB R2016a.

Preparation of Maximum Absorption Spectra

A 1 mL aliquot of profenofos LIB (2500 µg/mL) was diluted to 10 mL with methanol to obtain 250 µg/mL; similarly, 1.75 mL of diazinon LIB II (200 µg/mL) and 1.5 mL of chlorpyrifos LIB II (200 µg/mL) were each diluted to 10 mL to yield 35 µg/mL and 30 µg/mL solutions. All solutions were homogenised and scanned at 200–400 nm.

Preparation of Ratio Absorption Spectra

The MCR procedure resolved the overlapping spectra by sequential ratio division using predetermined divisors, followed by mean-centering to eliminate constant spectral effects[16]. Profenofos, diazinon, and chlorpyrifos spectra were divided using diazinon/chlorpyrifos, chlorpyrifos/profenofos, and diazinon/profenofos, respectively. Working ranges were 150–450 µg/mL (200–290 nm), 15–60 µg/mL (200–270 nm), and 15–50 µg/mL (200–250 nm). All measurements were performed in triplicate (n = 3) for reproducibility.[16]

Calibration Curve Development

The spectra processed through mean-centering amplitudes of the secondary ratio spectra for each analyte were correlated with their respective concentration levels to construct regression plots. Linear regression was performed to obtain the regression equation ($y = bx + a$) as indicated by the correlation coefficient (r), where good linearity was defined as $r \geq 0.997$.[17]

Method Validation

Method validation followed ICH Q2(R1) guidelines and comprised the following validation parameters:
Linearity: Evaluated from five concentration levels for each analyte (n = 3).

Limit of Detection (LOD) and Limit of Quantification (LOQ)

$$LOD = 3\sigma/b \quad LOQ = 10\sigma/b \qquad (1)$$

In which σ indicates the standard deviation associated with the blank signal, and b represents the slope of the calibration relationship.[17]

Precision

Assessed as the relative standard deviation (RSD) obtained from replicate analyses:

$$RSD = \frac{SD}{\overline{X}} \times 100\% \qquad (2)$$

An RSD < 2% was considered acceptable.[16]

Accuracy

Determined by the standard addition method at three spiking levels (80%, 100%, 120%) in triplicate (n=3). Per cent recovery (%) was calculated as:

$$\%Recovery = \frac{C_f - C_A}{C_A^*} \times 100\% \qquad (3)$$

Where C_f = final concentration after spiking, C_A = initial concentration, and C_A^* = added concentration.

Sample Analysis

Sample extracts were scanned at 200–400 nm and processed using the same divisor spectra and MCR steps as the standards. The mean-centered amplitudes from the second-ratio spectra were then inserted into the regression equations to quantify profenofos, diazinon, and chlorpyrifos residues.[16]

Flowchart Mean Centering of Ratio Spectra (MCR)

The MCR method resolves overlapping UV–Vis spectra by applying a sequence of mathematical transformations that enhance spectral selectivity. In this approach, the recorded spectra of the pesticide mixtures are first divided by an appropriate divisor spectrum to generate ratio spectra, effectively minimising the shared absorbance contributions responsible for spectral interference. These ratio spectra are then subjected to mean-centering, a transformation that removes constant and proportional components while amplifying variations attributable to each analyte. As a result, MCR produces analytical signals that are directly proportional to concentration and largely free from the distortions caused by overlapping chromophores. This enables selective and accurate quantification of multiple pesticides within a single measurement. The complete analytical workflow is summarised in Fig.-1.

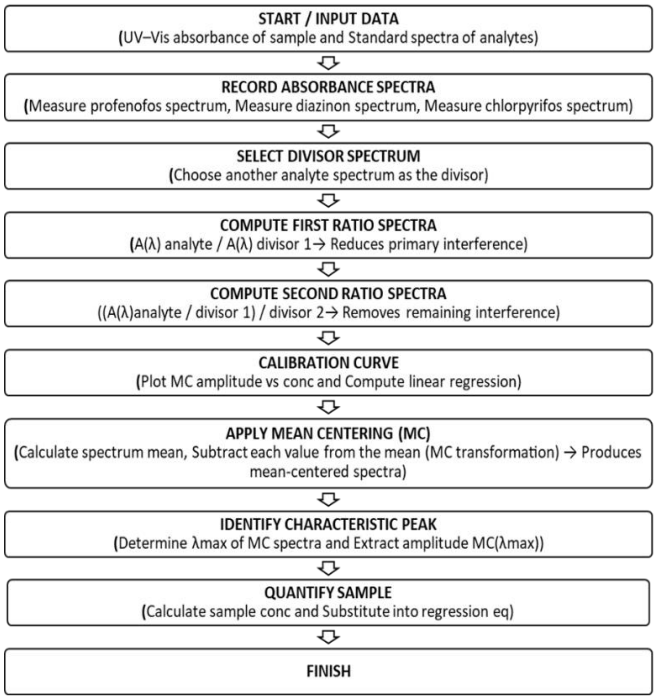


Fig.-1: Workflow of the MCR Method

The MCR workflow systematically reduces spectral overlap through the generation of ratio-based spectra and subsequent mean-centering. This dual transformation isolates characteristic MC signals (MC λ_max) that serve as calibration points in constructing regression models for each analyte. These MC signals form the basis for reliable multicomponent quantification without requiring chromatographic separation or further sample pretreatment.

RESULTS AND DISCUSSION

Ratio Absorption Spectra for Profenofos, Diazinon, and Chlorpyrifos

The absorbance spectra of profenofos, diazinon, and chlorpyrifos (200–400 nm) showed λmax at 276, 246, and 289 nm, respectively. These values agree with previously reported data, where profenofos exhibited maxima at 276 nm in methanol and 277 nm in ethyl acetate[18,19], diazinon at 246 nm in water[20]; and chlorpyrifos at 289 nm in ethyl acetate and 290 nm in methanol.[18,19]

Figure-2 illustrates the individual and overlaid spectra. The combined profiles reveal substantial spectral overlap, particularly in the 210–280 nm region, where the absorbance bands of the three analytes intersect. This overlap leads to intensified signals and significant mutual interference, making direct quantification by conventional UV–Vis spectrophotometry impractical. Because the absorbance at selected wavelengths is not uniquely attributable to a single compound, classical zero-order measurements cannot provide selective or accurate multicomponent analysis. This pronounced spectral interference underscores the need for mathematical approaches such as zero-crossing derivative spectrophotometry, Mean Centering of Ratio (MCR), and PLS chemometrics, which enhance spectral resolution and isolate analyte-specific

information. These techniques enable selective quantification despite extensive overlap, thereby overcoming the limitations of traditional UV–Vis methods.

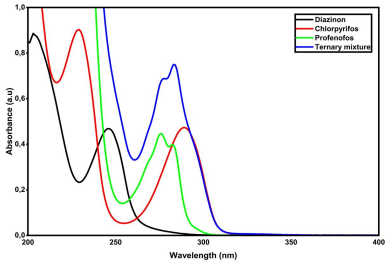


Fig.-2: UV–Vis Absorption Spectra and Maximum Wavelengths of Profenofos, Diazinon, and Chlorpyrifos

Ratio Absorption Spectra for Profenofos, Diazinon, and Chlorpyrifos

Each analyte spectrum was normalised against the spectra of the other components to extract its individual signal. Divisors were selected based on their spectral stability, absence of near-zero absorbance regions, and placement within the linear dynamic range, thereby preventing noise amplification during the division process. This strategy aligns with recent advancements in ratio-spectra and mean-centering methodologies, which highlight the importance of optimal divisor selection for generating smooth, well-defined spectral profiles.[16,21,22] Under these optimised conditions, the procedure yielded clean and distinctly separated ratio spectra, with spectral overlap progressively minimised across transformations, as illustrated in Fig.-3 to 5.

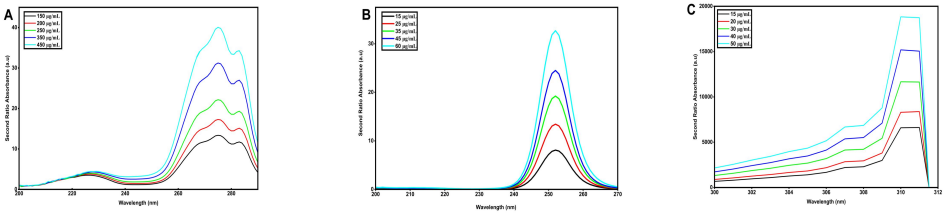


Fig.-3: Second-Stage Ratio-Transformed Absorption Spectra of Profenofos (a), Diazinon (b) and Chlorpyrifos (c)

Profenofos spectra were sequentially processed using diazinon (60 µg/mL) and chlorpyrifos (50 µg/mL) as divisors, whereas diazinon spectra were normalised with chlorpyrifos (50 µg/mL) followed by profenofos (350 µg/mL). For chlorpyrifos, diazinon (60 µg/mL) and profenofos (350 µg/mL) were applied in the same two-stage sequence. The use of these concentration-stable divisors ensured reliable ratio transformations and prevented noise enhancement during spectral division. This sequential processing effectively reduced the mutual spectral contribution of each compound, yielding second-ratio spectra with improved resolution and minimal overlap, an outcome consistent with the refinements reported for multi-step ratio-spectra techniques.[22–24] The clarified spectral patterns obtained from this two-step procedure were subsequently subjected to mean-centering, producing distinct MCR amplitudes. These mean-centered signals, which displayed linear concentration dependence, served as the analytical responses for calibration and quantification, in agreement with earlier successful implementations of the MCR method in multicomponent UV–Vis analysis.[14,25]

Result of MCR

The MCR procedure was subsequently employed to obtain the final analytical amplitudes for profenofos, diazinon, and chlorpyrifos. Mean-centering was carried out on the second-ratio spectra within the wavelength regions that provided the greatest selectivity for each analyte: 200–290 nm for profenofos, 200–270 nm for diazinon, and 300–350 nm for chlorpyrifos (Fig.-4). These optimised intervals ensured that the mean-centered signals reflected the true concentration-dependent responses of each pesticide with minimal spectral interference.

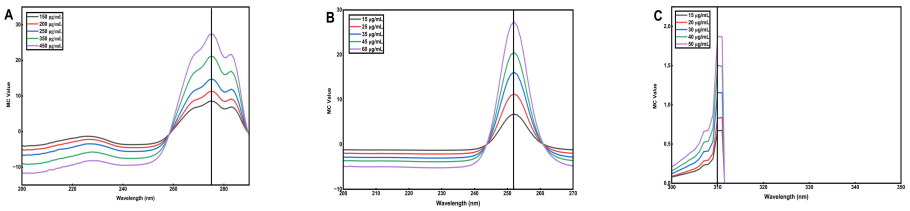
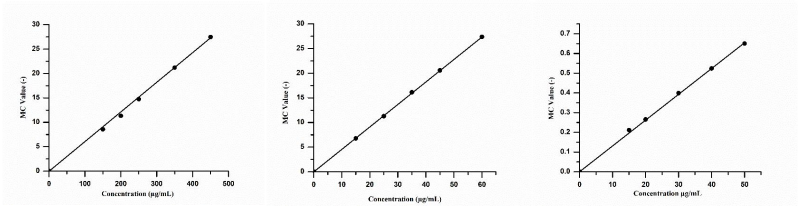


Fig.-4: Mean Centering of Ratio (MCR) Spectra of Profenofos (a), Diazinon (b), and Chlorpyrifos (c)

Figure-4(A–C) presents well-defined and non-overlapping MCR peaks for profenofos, diazinon, and chlorpyrifos at 275, 252, and 310 nm, respectively, which were selected as calibration wavelengths. The mean-centered spectra exhibited cleaner baselines and superior signal-to-noise characteristics compared with the raw or first-ratio spectra. This improvement is in line with previous reports demonstrating that the MCR transformation enhances spectral discrimination even in the absence of chromatographic separation.[14,25] The resulting mean-centered amplitudes were subsequently employed as the analytical signals for both calibration and method validation.

Method Validation

The calibration plots generated using the MCR-derived amplitudes demonstrated outstanding linearity and reproducibility for all three pesticides (n = 3). The regression profiles exhibited minimal dispersion around the fitted lines (Fig.-5), reflecting the stability of the analytical signals. As summarised in Table-1, the MCR transformation effectively reduced spectral interference and enabled the extraction of analyte-specific responses, thereby supporting reliable quantitative determination of profenofos, diazinon, and chlorpyrifos



A B C

Fig.-5: Calibration plots derived from Mean Centering of Ratio (MCR) Spectra for Profenofos (a), Diazinon (b), and Chlorpyrifos (c)

Table-1: Evaluation of Linearity and Sensitivity for the Developed MCR–UV Method

Parameter	Profenofos	Diazinon	Chlorpyrifos
Calibration equation	y = 0.0615x + 0.4588	y = 0.4578x - 0.0451	y = 0.0362x - 0.0662
Slope	0.0615	0.4578	0.0362
Intercept	0.4588	-0.0451	-0.0662
Linearity (r)	0.9986	0.9999	0.9973
LOQ (µg/mL)	64.7480	2.3176	14.8619
LOD (µg/mL)	19.4244	0.6953	4.4586

All correlation coefficients exceeded the minimum threshold specified by ICH Q2(R1) ($r > 0.997$), supporting the high linearity and selectivity typically associated with MCR-based calibration, as previously reported in related studies.[16,26] Method validation performed according to international guidelines[27], confirmed that the linear responses were acceptable, with limits of detection and quantification determined utilising well-established LOD and LOQ values that were determined based on the standard deviation (σ) and the calibration slope (S), employing factors of 3.3 and 10, respectively.[28] All three pesticides also fulfilled CIPAC acceptance criteria ($r \geq 0.99$),[29] and the results were consistent with earlier pesticide analyses that similarly demonstrated strong linear behavior.[30,31] The method exhibited good analytical sensitivity, yielding LOD/LOQ values of 19.4244/64.7480 µg/mL for profenofos, 0.6953/2.3176 µg/mL for diazinon, and 4.4586/14.8619 µg/mL for chlorpyrifos, all of which

meet established detection performance requirements.[32] Accuracy evaluation (Table-2) further showed close agreement between measured and fortified concentrations, confirming the quantitative robustness and reliability of the MCR–UV–Vis approach.[33]

Table-2: Accuracy and Precision Evaluation of the MCR UV–Vis Method at Three Fortification Levels

Level (%)	Spiked Concentration (µg/mL)	Net Spiked Concentration (µg/mL)	Mean Recovery (%)	%RSD
80	40.00	42.42 ± 1.12	106.06 ± 2.80	2.64
100	50.00	51.67 ± 0.04	103.56 ± 0.31	0.30
120	60.00	64.75 ± 0.92	107.92 ± 1.54	1.42

The recovery results at the 80%, 100%, and 120% fortification levels (106.06%, 103.56%, and 107.92%, respectively) fell within the generally accepted range of 70–120% and complied with the precision limits set by the European Commission (RSD ≤ 20%). Although the recoveries were slightly above the nominal values, suggesting a mild positive bias, supplementary measurements (99.00–101.20%; RSD < 2%) indicated that no significant analyte loss or matrix-induced suppression occurred. Collectively, these findings confirm that the method provides accurate and precise quantification across the tested concentration levels and satisfies the performance requirements of ICH Q2(R1), AOAC guidelines, and European Commission regulations.[27,28]

CONCLUSION

This study demonstrates that the MCR UV–Vis spectrophotometric approach provides a reliable, selective, and operationally simple approach enabling the concurrent quantification of profenofos, diazinon, and chlorpyrifos in ternary mixtures. The developed procedure fulfilled all major validation requirements, exhibiting excellent linearity, satisfactory accuracy, good repeatability, and low limits of detection and quantification. These performance characteristics confirm that the method is well-suited for multicomponent pesticide analysis, even in systems where severe spectral overlap would normally impede direct UV–Vis measurements. The MCR algorithm proved highly effective in resolving the overlapping absorbance profiles of the three organophosphates. Through sequential ratio spectra generation and mean-centering transformation, the method successfully isolated analyte-specific signals without the need for chromatographic separation or complex sample preparation. This capability reinforces the practical value of MCR for laboratories seeking simple yet robust alternatives to instrumental chromatography. Overall, the findings emphasise the analytical robustness and practical advantages of the MCR–UV–Vis method. Its rapid execution, minimal solvent consumption, and cost-efficiency make it a strong candidate for routine monitoring of multiresidue pesticide mixtures, particularly in settings where accessible and sustainable analytical tools are required.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and Well-being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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